U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-263 909

Pulmonary Cell Populations in Hamsters Maintained under Egyptian Laboratory Conditions

Al-Azhar Univ, Cairo (Egypt)

Prepared for

Environmental Monitoring and Support Lab, Las Vegas, Nev

Jan 77

EPA-600/3-77-006
January 1977

PB 263 909

Ecological Research Series

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/3-77-006		

4. TITLE AND SUBTITLE
PULMONARY CELL POPULATIONS IN HAMSTERS MAINTAINED UNDER EGYPTIAN LABORATORY CONDITIONS

5. REPORT DATE
January 1977

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
A.S. El-Sheikh, G.A. Abdel-Kader and S.O. Amin (block 9); and R.E. Stanley (block 12)

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Al-Azhar University
Cairo, Egypt

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.
Contract No. 03-546-1

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Monitoring and Support Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Las Vegas, Nevada 89114

13. TYPE OF REPORT AND PERIOD COVERED
Progress-Calendar Year 1974

14. SPONSORING AGENCY CODE
EPA/600/07

15. SUPPLEMENTARY NOTES
This study was supported by the Special Foreign Currency Program, P.L. 480.

16. ABSTRACT

This study was conducted to obtain baseline values for pulmonary cells in golden hamsters (*Mesocricetus auratus*) bred and maintained under the laboratory conditions of Al-Azhar University in Egypt. An improvised technique is presented for measuring pulmonary cells obtained by lung lavage in golden hamsters. The results of using this technique revealed a positive correlation between the total count of pulmonary cells and the body weight of the hamsters. Cell differential counts showed that more than 99 percent of the pulmonary cells were macrophages, with lymphocytes as the remainder. The findings are discussed and compared to those reported in the available literature.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
lung cell morphology phagocytes laboratory animals	pulmonary alveolar macrophages lung lavage hamster	06 C, P 14 B

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	9
	20. SECURITY CLASS (This page)	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (9-73)

EPA-600/3-77-006
January 1977

PULMONARY CELL POPULATIONS IN HAMSTERS MAINTAINED UNDER EGYPTIAN LABORATORY CONDITIONS

by

A. S. El-Sheikh, G. A. Abdel-Kader and S. O. Amin
Al-Azhar University
Cairo, Egypt
and
R. E. Stanley
Monitoring Systems Research and Development Division
Environmental Monitoring and Support Laboratory
Las Vegas, Nevada 89114

Contract No. 03-546-1

R. E. Stanley
Project Officer

Monitoring Systems Research and Development Division
Environmental Monitoring and Support Laboratory
Las Vegas, Nevada 89114

U. S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
LAS VEGAS, NEVADA 89114

DISCLAIMER

This report has been reviewed by the Environmental Monitoring and Support Laboratory-Las Vegas, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

INTRODUCTION

It has been established that inhalation of various air pollutants influences the number and function of pulmonary alveolar macrophages (PAM) (1-3). The PAM constitute the overwhelming majority of the pulmonary cell population and play an important role in the defense mechanism of the lung against infection.

This study was conducted to obtain baseline values for pulmonary cells in golden hamsters (*Mesocricetus auratus*) bred and maintained under the laboratory conditions of Al-Azhar University. The data will be used for comparison to that obtained from future studies concerned with the pulmonary effects in hamsters following exposure to various inorganic air pollutants indigenous to the Egyptian environment.

SUMMARY

In this study an improvised technique is presented for measuring pulmonary cells obtained by lung lavage in golden hamsters (*Mesocricetus auratus*). The results of using this technique revealed a positive correlation between the total count of pulmonary cells and the body weight of the hamsters. Cell differential counts showed that more than 99 percent of the pulmonary cells were macrophages, with lymphocytes as the remainder. The findings are discussed and compared to those reported in the available literature.

RESULTS

The absolute cell count (cells/mm^3) and the calculated relative cell count (cells/100 g body weight) obtained for each of the nine hamsters by the technique described in this study are shown in Table 1.

The average differential count for the nine hamsters was 99.34 percent PAM and 0.66 percent leucocytes.

The normal PAM harvested in the lung lavage varied in shape. The cell diameters ranged from 12 to 30 micrometers (μm), and the diameters of the nuclei ranged from 9.6 to 13.2 μm. As shown in Figure 1, the nuclei are deeply stained and eccentrically located. The cytoplasm is basophilic and shows ingested dust particles in several cells. In Figure 2, it is shown that the cell contours vary from oval to irregular, and occasional cytoplasmic processes are also seen.

MATERIALS AND METHODS

Nine male golden hamsters selected from breeding stock obtained from the Naval American Medical Research Unit at Abbassia, Egypt, and from the

TABLE 1. ABSOLUTE AND RELATIVE TOTAL COUNTS OF PULMONARY CELLS IN NINE GOLDEN HAMSTERS

Hamster Number	Number of Replicate Counts from Stock Suspension	Body Weight (g)	Mean Absolute Count (cells/mm^3)	Calculated Relative Count (cells/100 g body weight)
1	11	60	874	1457
2	9	80	1043	1304
3	6	77	1140	1481
4	6	75	1068	1424
5	5	170	2410	1418
6	4	170	2000	1176
7	3	170	2800	1647
8	7	170	2657	1563
9	4	170	2575	1515
	Average	127	1841	1443

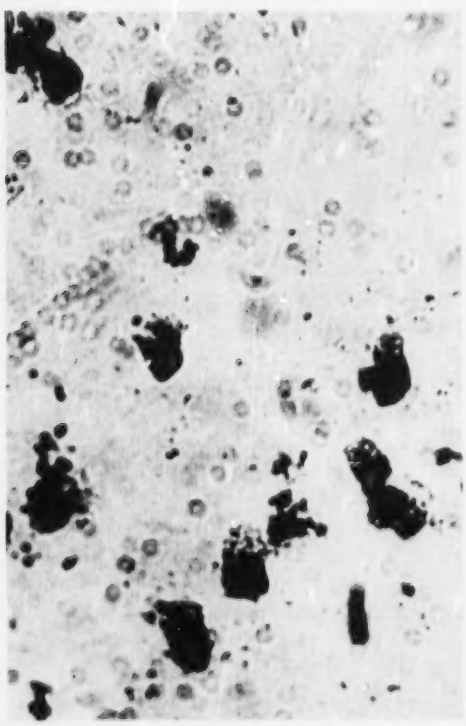

Figure 1. Cell nuclei deeply stained and eccentrically positioned. 280X.

Figure 2. Oval cells with occasional visible cytoplasmic processes. 320X.

Ministry of Health Laboratory for Serum Production at Helwan, Egypt, were used in the study. The hamsters were housed individually in galvanized wire cages under standard conditions of room temperature (22±1°C) and relative humidity (50±5%). The ration fed to the animals consisted of a mixture of fresh and commercially processed food containing approximately 20 percent protein, with water available *ad libitum*. Body weights ranged from 60 to 170 g.

The technique used for harvesting the alveolar pulmonary cells is a modification of those used by Myrvik *et al.* (4) and by Coffin *et al.* (5).

The hamsters were anesthetized by intraperitoneal injection of sodium pentobarbital (6.5 mg/100 g body weight). The trachea was exposed and incised slightly posterior to the larynx. A polyethylene cannula was inserted into the trachea and maintained in place with an annular ligature. Necessary precautions were taken to avoid any contamination by blood within the trachea or lungs. Three to four ml of normal saline (0.9 percent NaCl maintained at 37°C) with a few drops of gentian violet added to stain the nuclei of the cells to make them visible, were injected through the cannula into the lungs and allowed to remain for 15 minutes. The lavage fluid was then withdrawn from the lungs, using a syringe, and ejected into a graduated conical-shaped centrifuge tube which was positioned in a crushed-ice bath. The lung washing was repeated five more times in a similar manner, except that the lavage fluid was withdrawn from the lung immediately after each instillation.

The lavage fluid collected in the centrifuge tube from the six washings was centrifuged at 1500 rpm for 20 minutes. Following the centrifuging, the supernatant fluid was removed using a capillary pipette. The remaining cell pellet was then resuspended in 3 ml of normal saline and the mixture agitated by inverting the centrifuge tube until the cell pellet was no longer visible. The cell suspension was recentrifuged for 15 minutes, and the supernatant fluid was again removed with a capillary pipette. After this washing, the cell pellet was resuspended in 1 ml of normal saline using a gentle current of air as the source of agitation to effect the suspension. This suspension was used as the stock suspension for the total and differential counts and for the source of macrophages studied in the purified form.

The total cell count was accomplished using exactly the same procedure as that used for counting white blood cells. The cell suspension (stock suspension) was aspirated into a hemocytometer pipette and diluted (10 to 1) with normal saline containing a few drops of gentian violet. One drop was then placed on the counting chamber, covered with a cover slip, and the cells counted. After several replicate counts, the mean count (cells/mm^3) was used to calculate the total number of cells in the stock suspension. This absolute cell count was then used to calculate the relative count (cells/100 g body weight).

The cell differential count was made from the stock suspension, also. One drop of the stock suspension was spread over a clean glass slide, air dried, fixed with formalin vapor, and stained with Giemsa stain. The different types of cells (PAM, lymphocytes and neutrophils) were counted in each field and tabulated as to the relative percentage observed.

DISCUSSION

This study shows that the absolute pulmonary cell count of the stock suspension seems to vary directly with the body weight, and that the relative count calculated according to body weight was more or less constant. This finding indicates a positive correlation between total pulmonary cells, obtained by lung lavage, and body weight. This correlation is logical when considering the relationship between alveolar surface area and body mass.

As can be discerned, these apparent conclusions were drawn without benefit of any formal statistical analysis and appear obvious. Although the concept of the absolute cell count as a function of body weight is clearly established, the data were analyzed to confirm these obvious conclusions using a modified analysis-of-variance program considering all hamsters combined and all possible comparisons of all pair combinations. Testing the hypothesis of no difference among hamsters when the cell count is adjusted for body weight, the probability of this hypothesis being true changes from 3×10^{-24} percent to 4.4 percent, a 1.5×10^{24}-fold improvement in the consistency of the data due to the weight adjustment. Therefore, the improvement is so gross and obvious that statistical analysis was, in this case, superfluous. However, the authors are the first to admit that the number of animals used was less than desirable. The almost total absence of body weights in the 80-to 170-g range does not permit one to definitely establish the functional relationship indicated.

Myrvik *et al.* (4) using their own technique to obtain PAM in a high state of purity, reported that in the rabbit, the total yield from normal lungs usually varied between 0.1 and 0.2 ml of packed cells (8 to 16 million cells). Coffin *et al.* (5), however, reported the number of cells obtained from unexposed rabbits' lungs as high as 43.5 million with a standard error of 4.7 million. The rabbits used by these two groups of investigators were more or less the same weight. Nevertheless, the obvious discrepancy in their findings could be attributed to the different techniques employed by the two groups of workers, strain differences in the animals, and/or differences in individual laboratory conditions.

This work, however, presents a method of relating cell count to body weight, which tends to greatly minimize the differences reported when based on absolute count alone.

PAM constituted more than 99 percent of the total pulmonary cells recovered from the lung by lavage. Less than 1 percent of the recovered pulmonary cells were lymphocytes. Polymorphs and eosinophils were difficult to find in any of the smears.

By comparison, Gardner *et al.* (6) found that in the unexposed rabbit, 98 percent of the pulmonary cells were mononuclear macrophages. Small lymphocytes constituted 1 percent of the total observed, while occasional polymorphonuclear leucocytes and eosinophils comprised the remaining 1 percent. Myrvik *et al.* (4) reported that in the rabbit, also, the cell suspension contained less than 1 percent polymorphonuclear cells, with the alveolar macrophages constituting essentially all of the cells observed. Myrvik *et al.* (4) further stated that lymphocytes were seldom seen.

In smears stained with hematoxylin and eosin, Myrvik *et al.* (4) described the general morphology of alveolar macrophages obtained from rabbits' lungs as resembling plasma cells rather than blood monocytes. They further reported variation in size of the macrophages, although the cells possessed a strikingly uniform appearance. In this study, the Giemsa-stained material showed the PAM to vary both in size and uniformity. In fact, variations in shape and contour among the cells were even more pronounced than the variation in size, and protoplasmic processes were easily seen in some cells. None of the cells examined were binucleated or trinucleated as was reported by Myrvik *et al.* (4).

REFERENCES

1. Gardner, D. E., T. R. Lewis, S. M. Alpert, and D. L. Coffin. "The Role of Tolerance in Pulmonary Defense Mechanisms." *Arch Environ Health* 25. 1972.

2. Waters, M. D., D. E. Gardner, and D. L. Coffin. "Cytotoxic Effects of Vanadium on Rabbit Alveolar Macrophages In Vitro." *Toxicol Appl Pharmacol* 28. 1974.

3. Coffin, D. L., and D. E. Gardner. "Interaction of Biological Agents and Chemical Air Pollutants." *Ann Occup Hyg* 15. 1972.

4. Myrvik, Q. N., E. S. Leake, and B. Fariss. "Studies on Pulmonary Alveolar Macrophages from the Normal Rabbit: A Technique to Procure Them in A High State of Purity." *J Immunol* 86. 1961.

5. Coffin, D. L., D. E. Gardner, R. S. Holzman, and F. J. Wolock. "Influence of Ozone on Pulmonary Cells." *Arch Environ Health* 16. 1968.

6. Gardner, D. E., R. S. Holzman, and D. L. Coffin. "Effects of Nitrogen Dioxide on Pulmonary Cell Population." *J Bacteriol* 98. 1969.